Federal Ministry of Health, Nigeria

Laboratory Based HIV Rapid Test Validation in Nigeria
Phase 1
April 2007

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Conducted by

The Nigeria HIV Rapid Test Evaluation Working Group

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Federal Ministry of Health, Nigeria (FMOH)
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FOREWORD

In order to meet the Presidential mandate of placing 250,000 People Living with HIV and AIDS on treatment, the need to increase service uptake, particularly, in the rural areas has become imperative. Such expansion is not without challenges. One such challenge is a situation where the use of sophisticated equipment and storage facilities may not be available. It was in view of this that the rapid test kits (particularly the non-cold chain dependent ones) became necessary.

As a result of the importance of HIV testing, not only for HIV Counseling and Testing but also for Prevention of Mother to Child Transmission, emergency blood transfusions and clinical diagnosis, the need for well evaluated and reliable testing products whose performance and use are quality assured is essential and urgent.

To this end, a multi-agency HIV Test Kit Evaluation Working Group has been charged with the responsibility of evaluating HIV rapid test kits and making recommendations to government on the test kits to be included in the new HIV testing Algorithm.

Although an interim parallel testing algorithm is currently in use in Nigeria, the result of this evaluation exercise showed that there is no difference in accuracy between the parallel and serial algorithms.

I hereby endorse the recommendations of the Technical Working Group and approve that the results of this report be used for assessing the HIV status of Nigerian.

Dr. Shehu Sule MFR, mni
Acting Permanent Secretary
Federal Ministry of Health

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The Federal Ministry of Health wishes to congratulate the HIV Rapid Test Kit Evaluation Working Group on the successful planning and completion of the first phase of the HIV Test Kit Evaluation exercise.

We extend our profound gratitude to all organizations and persons who have contributed to the success of the exercise including the National Agency for the Control of AIDS, the National Agency for Food and Drug Administration and Control, and the Nigeria Institute for Pharmaceutical Research.

Our special thanks go to the Centres for Disease Control and Prevention-Global AIDS Programme (CDC-GAP) for providing financial and technical support for the implementation of the activity.

We also acknowledge the contributions of the staff and management of Asokoro Training Laboratory in anchoring the Laboratory component of the exercise

Dr. Ngozi Njepuome
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INTRODUCTION TO THE REPORT

HIV Counseling and Testing is the acknowledged entry point to comprehensive HIV Treatment, Care and Support. In countries with considerably high HIV prevalence it is advisable that all persons exposed to HIV infection should ascertain their HIV status.

With the widespread availability of Anti-Retroviral Therapy persons who test positive for HIV can assess early treatment, care and support while persons who test negative can access the many options open for non-risky behaviour.

To ensure unrestricted access to quality assured HIV testing, Government plans to adopt an HIV Testing Algorithm that is sensitive, specific and accessible to the majority of its citizens.

This report is the product of painstaking work and provides valuable scientific data to guide the choice of a National Testing Algorithm. It will be widely disseminated to all stakeholders.

Dr. Henry Akpan
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Federal Ministry of Health.

ABBREVIATIONS

ANC	Antenatal Clinic
CDC-GAP	Centers for Disease Control and Prevention, Global AIDS Program
EIA	enzyme immunoassay
EQA	external quality assurance
FMOH	Federal Ministry of Health, Nigeria
GON	Government of Nigeria
HCT	HIV counseling and testing
HIV	Human Immunodeficiency Virus
IHVN	Institute of Human Virology, Nigeria
IRB	institutional review boards
NACA	National Agency for the Control of HIV and AIDS
NAFDAC	National Agency for Food, Drug Administration and Control
NASCP	National AIDS and STIs Control Programme
NGO	Non-Government Organization
NIPRD	National Institute for Pharmaceutical Research and Development
PEPFAR	U.S. President's Emergency Plan for AIDS Relief
PMTCT	prevention of mother-to-child transmission
RTK	Rapid Test Kit
WB	Western Blot
WHO	World Health Organization

EXECUTIVE SUMMARY

The Government of Nigeria (GON), in collaboration with several development partners, in 2005, established a multi-agency working group charged with the responsibility of evaluating HIV rapid test kits and recommending their appropriate use at points of services in Nigeria.

The working group, using an internationally standardized method, created a set of criteria which guided the selection of nine HIV rapid test kits for evaluation. One major criterion for kit selection was non-reliance on refrigeration. Test performance was assessed in a one week laboratory exercise to determine sensitivity, specificity and operational characteristics of individual tests. A panel of well characterized sera collected from the different geo-political zones in Nigeria was used to assess kit performance.

Based on their characteristics and performance, six of the tests were selected for further evaluation, three test kits were dropped from further consideration due to poor performance, cost or complexity. The remaining six kits (Bundi, Determine, Double Check Gold, StatPak, SureCheck and UniGold) all had 100% sensitivity and high specificity, ranging from 97.9 to 100%.

In practice individual tests are not used for diagnosis of HIV; tests are used in combinations (algorithms) to increase diagnostic accuracy. Since a single specimen panel was used in this evaluation various algorithms could be proposed and the sensitivity/specificity of these algorithms calculated. Using the six test kits listed above there are 120 possible serial test algorithms. All of these were 100% sensitive, specificity ranged from 99.1 to 100%.

Parallel algorithms were also proposed and when compared to serial algorithm there was no difference in accuracy. There was however, a substantial difference in cost. Serial algorithms, in general, are half the cost.

Based on the outcome of this evaluation the following recommendations are proposed:

1. Serial testing should be adopted for use in Nigeria. In this evaluation it was as accurate as and more cost effective than parallel testing.
2. Three possible serial test algorithms are proposed.
3. These algorithms are built around four tests which performed well in this evaluation, have a proven record of use in Nigeria, satisfy concerns for purchasing locally produced products and make use of kits soon to be available in large supply in Nigeria.
4. All HIV diagnostic testing, especially that using rapid tests, should be linked to a well developed training program and a comprehensive quality assurance system.
5. A formal evaluation of HIV rapid test performance is an ongoing process that begins prior to implementation of testing and continues after testing programs have been scaled-up in the field. This report provides data on a laboratory based validation of individual test products. Proposed algorithms should now be field tested and monitored.

INTRODUCTION

Nigeria is a highly-populated country of 140 million people with an HIV prevalence rate around 5% (Antenatal Clinic [ANC] Survey, 2005). It is a multi-ethnic society with a large proportion of the population living in rural settings (64%).

Traditionally, HIV testing has been the gateway to HIV/AIDS prevention, treatment, care and support. To date, many African countries have evaluated simple, rapid HIV testing as a tool for fighting the HIV epidemic. All of these studies have demonstrated that the use of rapid testing strategies can be an important part of overall HIV testing in resource-poor settings, where cold storage capacity, reliable power, efficient transportation and sufficient numbers of skilled laboratorians may not be readily available.

The use of HIV rapid testing has also dramatically increased the proportion of tested individuals who receive their results. Prior to the availability of rapid testing, same-day results were not available, and an estimated one-third of those tested did not return to learn their HIV status. The Government of Nigeria (GON) is currently working to expand quality HIV counseling and testing (HCT) services as a prevention intervention, and as an entry to care and treatment. Therefore, the need for well-evaluated, reliable testing products whose performance and use is quality-assured is essential and urgent.

GON health care facilities and non-government organizations (NGOs) in Nigeria are currently providing HIV rapid testing for HCT, prevention of mother-to-child transmission (PMTCT), emergency blood transfusions, and clinical diagnosis. When rapid testing is provided in settings where people learn their status, a multiple test algorithm is used. The U.S. President's Emergency Plan for AIDS Relief (PEPFAR) program supports HIV diagnosis through rapid testing, over the past six months more than 350,000 clients attending HCT sites and nearly 100,000 women attending PMTCT programs across Nigeria. In addition, test kits were provide for emergency HIV screening of 20,000 units of blood in the past year.

Enzyme immunoassays (EIA) and Western Blot (WB) technologies have been available in Nigeria; however cost and the required infrastructure have limited their availability. HIV rapid tests offer a cheaper, simpler and faster alternative. HIV Rapid Test Kits (RTKs) have been used in Nigeria for at least ten years. However, they have not always been used in a systematic or standardized fashion, such as a three-test algorithm as recommended by the World Health Organization (WHO). As of 2003, there was no national HIV rapid testing algorithm in Nigeria (though recommendations did exist in the initial VCT manual). Therefore, PEPFAR adopted the testing algorithm used successfully in past HIV ANC surveys. This was a serial algorithm, using Capillus (for screening) followed by Genie II (for all Capillus positive specimens), and Determine as a tie-breaker (in cases where discordant results were seen between the initial two tests).

The first two tests in this algorithm require refrigeration, and it was apparent that this would hinder expansion of HCT beyond tertiary and secondary healthcare facilities. In early 2006 a temporary move was made toward a non-cold chain dependent testing algorithm. Parallel testing was suggested using any two of the following tests: Determine, StatPak, Bundi, Double Check

Gold or Inocheck. None of these tests require refrigeration and have eased the burden of cold chain during transport to and storage at testing sites. These tests are also far easier to run and have allowed Nigeria to move toward the use of trained, non-laboratory staff for HIV diagnostic testing at HCT sites. To resolve discordant results Genie II or Capillus was suggested or clients could be referred to a higher level facility for EIA or WB testing. However, both tie-breaker tests require refrigeration, there were concerns over loss of clients and availability of EIA / WB testing. There still existed a need for a completely non-cold chain dependent algorithm.

No formal evaluation of HIV rapid tests for the development of an algorithm has been conducted in Nigeria. HIV rapid tests have been evaluated individually, but multiple test products have not been evaluated with a single, well-characterized specimen set representative of the entire country.

Project Goals
To evaluate the performance of HIV RTKs for the development of a single, national algorithm appropriate for diagnostic and non-diagnostic settings in Nigeria through evidence-based validation processes. This algorithm should be non-cold chain dependent, highly sensitive, highly specific and cost-effective.

To develop a list of highly sensitive and specific HIV rapid tests with documented good performance, which can be used as alternative tests in situations such as stock-outs of test in the primary algorithm.

METHODOLOGY

Establishment of an Evaluation Working Group
In August 2005 a multi-agency working group was developed by the GON for the evaluation of HIV RTKs. This included participation from the GON's Federal Ministry of Health (FMOH) and international donor organizations; specifically, National AIDS and STIs Control Programme (NASCP), National Agency for the Control of AIDS (NACA), National Agency for Food, Drug Administration and Control (NAFDAC), National Institute for Pharmaceutical Research and Development (NIPRD), World Health Organization (WHO), Centers for Disease Control and Prevention, Global AIDS Program (CDC-GAP) and some partners implementing the PEPFAR program in Nigeria with international experience in rapid test evaluations, Institute of Human Virology-Nigeria (IHVN). See appendix A.

Protocol Development
The protocol for this evaluation was developed from Guidelines for Appropriate Evaluations of HIV Testing Technologies in Africa, jointly developed by the CDC and the African Regional Office of the WHO (WHO/AFRO). This document outlines a three-phase strategy for evaluating HIV rapid tests and monitoring the performance of a testing algorithm.

The process is started with a laboratory-based evaluation using well-characterized specimens to determine test performance and results in proposed algorithms (Phase I). This is followed by a field evaluation using whole blood at a few points of service to gather data on human

performance, such as interpretation of results (Phase II). Once a new algorithm is established, it should be monitored indefinitely through a national external quality assurance (EQA) program (Phase III).

Test Selection Criteria

Currently there are dozens of HIV RTKs available commercially, but not all of these are appropriate for use in Nigeria. The following criteria were developed by the evaluation working group to decide which HIV RTKs should be evaluated. Criteria have been ranked by order of importance. The first five criteria were deemed by the working group to be the most important (presented below in bold text).

1) **Stability within the climate in Nigeria, and not dependent on cold chain**
2) **Ability to test whole blood**
3) **Easy to perform and interpret**
4) **Low test price**
5) **Ability of manufacturers to produce and provide adequate numbers of testing kits to meet the needs of testing programs in Nigeria**
6) Prior experience and validation - documented performance in Nigeria and other African countries
7) Ability to detect HIV-1, HIV-2 and HIV- type O subtypes
8) Ability to detect IgG and IgM antibodies to reduce the window period
9) Do not require additional equipment to run tests or read results
10) Packaging of test kits not excessively bulky
11) Long shelf life (at least one year) and robust
12) Test results provided in at least 30 minutes

There were seven HIV rapid tests which met these criteria. Two additional tests were included in this evaluation for the following reasons. OraQuick meets most of the inclusion criteria but is more costly than other tests ($4.00) and has a shorter shelf life (6 months). However, members of the working group expressed interest in evaluating this test since it has the capacity to test oral fluid and could be used in settings where oral testing is the only viable option. Bundi was also included; this is a new product and therefore does not have a documented performance record (criteria 6). The capacity of the manufacturer to produce adequate numbers of tests to meet the needs of programs in Nigeria is not known (criteria 5). Bundi was included in this evaluation since it is a locally assembled test product, an important issue for the GON. All evaluated kits are listed in Table 1 along with test characteristics. NAFDAC registration information is also provided for each test.

Principles of These Tests

All the tests studied in this evaluation are qualitative tests for the detection of antibodies to HIV-1 and HIV-2. All of these tests, with the exception of InstantChek, use immunochromatographic technology (also described as lateral flow). Recombinant and/or synthetic proteins representing the immunodominant regions of the envelop proteins of HIV-1 and -2 (such as glycoproteins gp41, gp120 and gp36) are immobilized in the test regions of a reaction strip (nitrocellulose). A small volume of sample (whole blood, plasma or serum) is added to the sample pad at one end of this strip. This pad acts as a filter to remove red blood cells or other blood solids (such as fibrin

clots) and provides a substrate for reconstitution and mixing of the sample with the colloid-antigen conjugate (including either selenium or gold as a colorimetric agent).

Some kits include a buffer which is added just after the specimen, this facilitates the flow of liquid through the strip. As the specimen and conjugate migrate through the strip to the immobilized recombinant antigens at the detection window, a red/purple line is formed if HIV specific Ab is present. If antibodies to HIV are absent, the antigen-selenium colloid flows past the patient window.

To ensure assay validity, a procedural control bar is incorporated in the assay strip, which provides an indication that a specimen has been added to the strip and that fluid is flowing adequately through the device. Typically, test results are interpreted within 15 – 20 minutes.

Many lateral flow strips (Bundi, DoubleCheck Gold, First Response, Stat-Pak and UniGold) are incorporated into a plastic cassette. This is then sealed in foil package to preserve the test from humidity. These cassettes allow for easier labeling and handling of the reaction strip. The manufacturers of SureCheck HIV have gone one step further and encased the strip into a clear plastic tube with a small capillary at one end for specimen loading. This tube completely encloses the strip, preventing contamination of the strip or exposure of the specimen to the tester, while at the same time allowing for easy viewing of the test results.

While OraQuick can test whole blood, plasma and serum, it was primarily designed to test oral fluid. To facilitate this, a collection pad is attached at one end of the device.

The makers of Determine do not use a cassette; instead test strips are attached to a flexible foil backing sealed with a foil cover. Ten tests are attached to create a card. One hundred tests are packed into a single envelope measuring 15 by 25 centimeters. This type of packaging greatly reduces the size and weight of test packages, which in turn reduces transport/shipping costs.

InstantChek uses a different test principle: it is a ‘flow through’ device. HIV antigens are immobilized on a membrane through which specimens are allowed to flow (on to an absorbent pad). If HIV-specific antibodies are present, they bind to the antigen and the addition of colloidal gold particles results in a red spot. A control spot is also incorporated into the membrane.

Source of Specimens

During the latter half of 2005 and the first half of 2006, a specimen panel was created from two sources: currently existing sample archives (left-over plasma or serum routinely collected for diagnostic purposes) in HIV testing labs at federally-administrated teaching hospitals, and remaining samples from a joint CDC / University of Maryland HIV seroconversion project. Efforts were made to ensure that specimens were contributed from sites in all six of the geo-political zones in Nigeria.

A total of ten facilities contributed specimens for this evaluation (a list of sites can be found in Appendix A), but specimens from only five facilities were used because specimens from some sites arrived too late for reference testing. Participating labs and institutions were instructed to remove all patient identification information from each specimen and report only the HIV sero-

status. All specimens included in this validation were unlinked and anonymized prior to inclusion; no blood was drawn for the sole purpose of this validation.

Care was taken to ensure that all validation specimens were of high quality. For this reason, all specimens included in this evaluation met the following criteria:

Properly collected, no hemolysis
Properly processed, no obvious signs of fungal or bacterial contamination/growth
Properly stored, at -20°C
Freshly collected specimen, not stored for longer than two months at the collection sites
Clear HIV Enzyme Immunoassay (EIA) sero-status, positive or negative
HIV positive specimens had to contain high titers of HIV-specific Ab, EIA signal to cutoff ratio of 3.0 or higher
For HIV negative specimens EIA results comparable to that for the kit negative control
Adequate specimen volume, at least 3 ml

Upon receipt, all specimens were given a new ID numbers, logged into a database and divided into two to three aliquots (to avoid repeated freezing and thawing, detrimental to Ab titers). All specimens were stored at -20ºC until characterization and evaluation. During the validation period specimen aliquots were kept at 4^{o}C.

Sample Size

The final specimen panel contained 528 specimens, of which 198 were HIV-1 positive and 330 HIV negative. A panel size larger than 500 provides a 95% confidence interval (± 2%) for calculating sensitivity and specificity.

Site for Laboratory-Based Validation

All laboratory work associated with this evaluation was carried out at the Asokoro Training Laboratory, located on the campus of the Asokoro General Hospital. This work included specimen characterization, storage and the evaluation exercise. This IHVN supported site was selected for the following reasons: current status as a national HIV laboratory training facility, central location with in Federal Capital Territory, constant electrical power, ongoing external quality assurance / laboratory monitoring program, appropriate infrastructure for reference testing (EIA equipment) and adequate specimen storage space at 4 and -20°C.

Reference Testing

In September of 2006 all specimens were fully characterized using standardized reference testing (gold standard), two EIAs and WB for all EIA reactive specimens. The use of WB allows for standardization and data sharing between different evaluations. Any specimens having discordant EIA or WB results were excluded from the panel. Specimens with indeterminate WB results were excluded.

The two EIAs selected for this validation were Vironostika HIV Uniform II Plus O (Biomerieux) and Genscreen (BioRad). Both assays are widely used throughout Africa, consistently produce clean data and detect HIV-specific Ab. An Ab-only test is the most appropriate for comparison with HIV rapid tests. The WB kit selected was New LAV blot I (BioRad). All reference testing was conducted per the manufacturer's instructions by CDC-Nigeria laboratory staff. Kit controls

and an in-house positive control (low positive developed in Nigeria) were included on all EIA plates. Controls were run with every batch of WB strips.

A portion (53 of 198) of the HIV positive specimens received additional testing to rule out HIV-2 co-infection, Genie II was used for this purpose.

Testing Procedure

After all specimens were characterized they were randomized and assigned new ID numbers between 1 and 528. The order of positive and negative specimens was mixed to allow for blinded testing. Ten laboratorians experienced in HIV serology from the ten sites which contributed specimens for the evaluation (Appendix A) were recruited for the evaluation. (See appendix B for list of laboratorians)Over a one-week period in December 2006, the laboratorians were provided background information on the evaluation, refresher training on Good Laboratory Practices and orientation to the data entry forms. Job aids were provided for each rapid test and each test was demonstrated by a CDC laboratorian. Under the supervision of CDC and NASCP laboratory staff, the laboratorians practiced on control specimens prior to evaluating the panel. Laboratorians worked in pairs, each pair evaluated approximately 100 specimens over a half day period per test product. Specimen sets were rotated between the laboratorians. Each test result was read by two laboratorians independently.

After each test product was evaluated all laboratorians completed a questionnaire concerning various aspects of the rapid test they had just evaluated (Appendix D). This focused aspects on running and reading test results, including ease of reading the reaction line, ease in interpreting the test results, ease in learning the test procedure and overall ease in running the assay; and packaging size and waste generation. This was done in an effort to capture information, in addition to accuracy, which is also critical in identifying tests for an algorithm.

Data Collection and Analysis

All test results were collected on paper forms (Appendix C) and entered into a spreadsheet database (MS Excel) for analysis. The sensitivity and specificity of each rapid test were calculated by comparing rapid test results with reference results derived from EIA/WB testing.

Calculation of Sensitivity and Specificity

	EIA Gold Standard Positive	**EIA Gold Standard Negative**	
Test Positive	**A**	**B**	**A + B**
Test Negative	**C**	**D**	**C + D**
	A + C	**B + D**	

Sensitivity = A/A+C

Defined as the ability of an assay being evaluated to correctly detect specimens containing Ab to HIV. In other words, sensitivity is the percentage of true positive HIV specimens identified by the assay under evaluation as positive (A), divided by the number of specimens identified by the reference assays as positive (A+C).

Specificity = D/B+D

Defined as the ability of an assay being evaluated to correctly detect specimens that do not contain Ab to HIV. In other words, specificity is the percentage of true negative specimens identified by the assay being evaluated as negative (D), divided by the number of specimens identified by the reference assays as negative (B+D).

In practice, individual tests are not used for the diagnosis of HIV in patients. International recommendations (WHO, Guidelines for Appropriate Evaluations of HIV Testing Technologies in Africa, page 4) support the use of multiple tests as part of a testing algorithm to improve the overall accuracy of diagnosis. In order to assess appropriate testing algorithms, this evaluation includes the calculation of sensitivity and specificity for all possible combinations of rapid tests in three test algorithms administered serially or in parallel. Not all test kits were deemed suitable for inclusion in further analysis of testing algorithms based on the following criteria: poor performance, cost or complexity of use.

Ethical Review and Approval
Prior to the evaluation, the protocol was reviewed and approved by the institutional review boards (IRB) of CDC-GAP and NIPRD.

RESULTS

Individual Test Results
The sensitivity and specificity results have been calculated for each individual test, as shown in Table 2. All nine tests performed well in this evaluation, as indicated by high sensitivity and specificity values. The sensitivity value for seven of the nine tests is 100%, indicating that none of these tests produced false negative results. Two tests, First Response and InstantChek, had lower sensitivities (98.99% and 96.97% respectively). Specificity varied slightly between the tests, ranging from 100% to 96.06%; OraQuick and Stat-Pak were each 100% specific.

Kits Dropped from Consideration
Due to test kit characteristics, performance or cost, three test products were removed from consideration for a testing algorithm. These are InstantChek, First Response and OraQuick.

InstantChek was one of the first 'rapid' tests designed for the detection of HIV and thus utilizes older technology. It was excluded for the following reasons:

1. InstantChek had the lowest test results of the nine products included in this evaluation (sensitivity 96.97% and specificity of 96.06%).
2. This test is much more complex to run than others tested. The kit includes eight components and solutions, some of which require reconstitution and storage at 4°C. The other tests included in this evaluation include only two components each: a test strip and a buffer.
3. The test procedure is complex. The tester must first decide between two different procedures, depending on the specimen type. Both procedures require the addition of various quantities of test solutions. Testing whole blood requires an additional step to filter out red blood cells. In fact this kit did not meet all of the evaluation criteria (specifically criteria 3, easy to perform and interpret).

4. This test received poor ratings on the questionnaire administered to the laboratorians. They ranked this test as the most difficult overall to run (Figure 1, D). They also ranked this procedure as the most difficult to learn (Figure 1, C).
5. This evaluation utilized frozen plasma; several specimens required multiple testing with InstantChek due to clogged membranes (specimens did not flow through the reaction membrane resulting in invalid results). Flow-through devices are more susceptible to clogging, especially with specimens which have been frozen.
6. The laboratorians also reported larger numbers of specimens presenting with high backgrounds (staining of the entire membrane by the colloidal gold) and difficulty in interpreting test results.

The manufacturers of First Response (Premier Medical Corporation) have made strides to produce a low-cost HIV rapid test, and of those products included in this evaluation it is the least expensive (US$ 0.55). Like all the other tests in this evaluation, First Response detects Ab to HIV-1 and HIV-2, however, it goes one step further and differentiates the infecting virus. This is done through reaction lines for each virus. All the HIV positive specimens included in this evaluation represent HIV-1 infections. However, of the 198 positive specimens, over one quarter (53) tested incorrectly as HIV-1 and -2 positive (dual infections) with First Response. In an HCT setting, this would cause confusion and require extensive supplemental testing to sort out the HIV sero-status of a large proportion of those with positive results.

OraQuick is capable of testing whole blood, plasma and serum, but was designed for testing additional specimen types, specifically oral fluid. This results in a more expensive test product. When compared to most other test products in this evaluation, it is between three and eight times more expensive. OraQuick also has a shorter shelf life (six months) than other tests.

Accuracy of Testing Algorithms

In diagnostic settings, rapid tests are used in testing algorithms, not as individual tests. One major advantage to evaluating RTKs using a single, well-characterized specimen panel is that sensitivity and specificity can be calculated for all possible combinations of tests. This has been done for both parallel (Table 3) and serial (Table 4) algorithms.

All possible parallel algorithms had a sensitivity of 100%, which indicates that none of the specimens in this panel has a false-negative result with more than one test product. Specificity was also high, ranging from 99.1% to 100%. This represents from three to zero false positive results for each algorithm. Over one third (24 of 60) of possible test combinations had the highest possible (100%) sensitivity and specificity.

For all 120 possible serial algorithms, sensitivity is 100%. Specificity ranged from 99.1% to 100%. Over half (67) of the proposed algorithms have 100% sensitivity and specificity. This includes five of the eight algorithms utilizing the two test kits currently in wide use in Nigeria (Determine and StatPak).

The number of specimens (out of 528) requiring a tie-breaker test due to discordant results between the first two tests is also reported for serial and parallel algorithms. Eight of 30 serial algorithms (two test combinations) do not required the use of a tie breaker testing. Other

combinations, for both algorithm types, ranged from one to ten, representing at most 2% of specimens.

Cost Estimates for Algorithms
The anticipated cost associated with each algorithm has been calculated. For parallel testing, the price of the initial two tests were added. The cost of the tie-breaker test was not included, since the frequency of use of a tie breaker is low (at most 1.8 % of the time).

For serial algorithms, an HIV prevalence of 10% was used. The full cost of the first test was added to 10% the cost of the second test (since the second test would only be used to confirm positive test results). Serial algorithms ranged in price from US$ 0.73 to US$1.91, while parallel algorithms ranged from US$ 1.49 to US$ 3.35. In general, serial algorithms are half the price of parallel algorithms since they require two tests to be run on all clients, even those 90% of clients who are HIV- negative.

RECOMMENDATIONS

1. HIV Oral Testing
OraQuick preformed extremely well in this evaluation with plasma/serum (100% sensitivity and specificity) and was rated by the laboratorians as having one of the easiest reactions lines to read (Figure 1, A). OraQuick should be considered as an option for specific populations or programs where traditional HCT diagnostic testing based on blood collection is not feasible. However, this should be linked to further evaluation of test performance with oral fluid. The short shelf life, 6 months, of this product needs to be considered to insure there is not increased wastage due to expired kits.

2. Limited Number of Test Algorithms
Many African countries fighting the HIV epidemic have found that diagnostic programs are stronger and produce more reliable results when a limited number of algorithms are selected and advocated for points of service. These algorithms should also be linked to a well-developed and distributed training program, particularly in light of the fact that most testing errors are caused by improperly running / reading of tests or specimen mix up. A comprehensive quality assurance program, which monitors all aspects of testing, should also be in place. Ideally this should monitor test kit performance from their arrival into Nigeria down to tester performance at individual sites.

3. Ongoing Evaluation
A formal evaluation of HIV test kit performance is an ongoing process that begins prior to implementation of testing and continues after tests have been implemented in the field. This report provides data on a laboratory-based validation of individual test products (phase I). Now that a select number of highly sensitive and specific test products have been identified they should be quickly field tested in various combinations (phase II). This can be coordinated centrally using a limited number of NGO supported HCT sites and tertiary facilities with appropriate lab infrastructure (EIA and WB capacity) already in place.

The HIV rapid test algorithms currently in place and those planned for future implementation in Nigeria should be continuously monitored through a quality assurance program (phase III) developed within Nigeria. This program should have the capacity to rapidly identify and correct testing problems related to the selected test kits and use of those kits in algorithms. Various tools currently exist which can be used in combination to support such a program.

4. Serial versus Parallel Testing

Conventional wisdom suggests that parallel testing is more accurate than serial testing. This and other evaluations suggest otherwise. Several serial testing options provided the highest possible sensitivity and specificity (100%) at nearly half the cost of parallel algorithms. Using a serial algorithm in Nigeria is consistent with WHO-recommended strategies for resource-poor settings.

5. Proposed Test Algorithms

Determine and StatPak have been used widely in Nigeria (Determine since the 2001 ANC survey and StatPak since 2006). Both tests have been evaluated and used internationally; Determine in particular is in wide use throughout Africa. In this evaluation both tests had high sensitivity and specificity individually and in serial algorithms. Determine with its high sensitivity (100%) is strongest as a screening test and should be used as the first test in any proposed algorithms. Determine is not recommended as a confirmatory test due to its lower specificity. Use as a tie-breaker is only recommended in the event that StatPak is not available.

Over the years, large numbers of laboratorians have been trained on both these kits as part of the national program. HIV/AIDS programs in Nigeria have already invested a great deal in these two tests; this includes not only the numbers of testers which have been trained but also the training packages which have been developed or adapted within Nigeria.

Laboratorians working with Determine in this evaluation reported that it is the most compact test kit, allowing for less expensive transport. They also reported that it generates the smallest amount of waste, alleviating some concerns about biohadzardous waste disposal at healthcare facilities (Figure 1, E and F). StatPak ranked high in the area of reaction line readability and results interpretation (Fig 1, A and B).

A serial algorithm using Determine and StatPak is cost effective, less than one US dollar. This is important when considering the size of the HCT program in Nigeria and the number of tests required to support it.

UniGold is also widely used internationally and performed well in this evaluation. In light of the fact that larger numbers of tests will soon be available in Nigeria to support HIV diagnostic testing programs, it also should be included.

The use of locally-manufactured test kits is a priority for Nigeria. Bundi, a locally-assembled test kit, performed well in this evaluation. As mentioned previously in this report, Bundi is a new test product and little is known about the consistency of test kit quality or capacity of the manufacturer to provide the numbers of test kits needed for the country. On the basis of its performance and recognizing the need to access factors such as ongoing quality assurance, adequacy of supply of kits and the development of a track record, it is recommended that Bundi

be included as a tie-breaker test. This would allow for continued monitoring of this new product. Meanwhile, the quality assurance in place during manufacturing should be documented. This would also allow the manufacturer sufficient time to scale up production.

Based on this evaluation, the following three algorithms are recommended:

SERIAL ALGORITHMS

Screening Test	Confirmation of Positives	Tie-breaker
Determine	**StatPak**	**Bundi**
UniGold	**StatPak**	**Bundi**
Determine	**UniGold**	**StatPak**

The limitations for this type of evaluation should be recognized. Six non-cold chain test kits (Bundi, Determine, Double Check Gold, StatPak, SureCheck and UniGold) performed well in this lab evaluation both as individual tests (Table 2) and in serial combinations (Table 4). Performance data from a phase II field evaluation is not currently available to inform a decision on a single algorithm. The data from this initial evaluation suggests that any combination of these six tests in three test, serial algorithms would perform well. Those tests with the highest sensitivity (such as Determine and UniGold) should be used as the screening test and those with highest specificity (such as StatPak) used for confirmation.

Table 1. Characteristics of HIV rapid tests included in this evaluation

	Bundi Rapid HIV 1/2	**Determine HIV-1/2**	**Double Check Gold HIV 1&2**	**First Response HIV 1-2.0**	**HIV 1/2 STAT-PAK Assay***	**InstantCHEK HIV 1+2**	**OraQuick Advance HIV-1/2**	**Sure Check HIV 1/2* (also called Clearview Complete)**	**Uni-Gold HIV****
Manufacturer	Bundi Interanational Diagnostics Ltd.	Abbott Laboratories	Orgenics, Ltd.	Premier Medical Corporation	Chembio Diagnostics Systems, Inc.	EY Laboratories	OraSure Technologies	Chembio Diagnostics Systems, Inc.	Trinity Biotech
Price per Test (US$)***	1.50	0.85	0.64	0.55	1.35	0.75	4.00	1.75	1.60
Tests per Kit	25	20 or 100	100	30	20	40 or 100	100	25	20
Cold Chain	No	No	No	No	No	No	No	No	No
Shelf Life (months)	12	14	12	15	18	18	6	12	15
Storage Temp (ºC)	4 - 30	2 - 30	2 - 30	4 - 30	8 - 30	-20 -28	2 - 27	8 - 30	2 - 27
Sample Type	whole blood, serum, plasma	whole blood, serum, plasma	whole blood, serum, plasma	whole blood, serum, plasma	whole blood, serum, plasma	whole blood, serum, plasma	whole blood, serum, plasma, **oral fluid**	whole blood, serum, plasma	whole blood, serum, plasma
Sample Volume (ul)	10 - 20	50	10	10 - 20	5	40	5	2.5	60
Assay Type	Immuno-chromatography	Immuno-chromatography	Immuno-chromatography	Immuno-chromatography	Immuno-chromatography	Immuno-chromatography	Immuno-chromatography	Immuno-chromatography	Immuno-chromatography
Solid Phase	Membrane, Lateral flow	Membrane, Lateral flow	Membrane, Lateral flow	Membrane, Lateral flow	Membrane, Lateral flow	Membrane, Flow through	Membrane, Lateral flow	Membrane, Lateral flow	Membrane, Lateral flow
NAFDAC Registered	03-0653	03-0622	03-0875	03-0925	03-0934	Not Registered	Not Registered	03-0935	03-1011
USAID Procurement Approved	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
US Food and Drug Administration (FDA) Approved	No	No	No	No	Yes*	No	Yes	Yes*	No**

* FDA approved with US-specific labeling
** The recombinent version of this test (Uni-Gold) is FDA approved
*** All pricing information obtained by Supply Chain Management System (SCMS) using a common methodology

Table 2. Sensitivity and Specificity Calculations for Individual Rapid Tests

TEST KIT **SEN / SPEC**

Bundi

	Gold Standard: True Positive	Gold Standard: True Negative			
Test Positive	**198**	**1**	199	Sensitivity	**100**
Test Negative	**0**	**329**	329	Specificity	**99.7**
	198	330			

Determine

	Gold Standard: True Positive	Gold Standard: True Negative			
Test Positive	**198**	**7**	205	Sensitivity	**100**
Test Negative	**0**	**323**	323	Specificity	**97.9**
	198	330			

Double Check Gold

	Gold Standard: True Positive	Gold Standard: True Negative			
Test Positive	**198**	**7**	205	Sensitivity	**100**
Test Negative	**0**	**323**	323	Specificity	**97.9**
	198	330			

First Response

	Gold Standard: True Positive	Gold Standard: True Negative			
Test Positive	**196**	**5**	201	Sensitivity	**99.0**
Test Negative	**2**	**325**	327	Specificity	**98.5**
	198	330			

However, of the 198 positive specimens 53 (over one quarter), tested as HIV-1 and -2 positive

Instant Chek

	Gold Standard: True Positive	Gold Standard: True Negative			
Test Positive	**192**	**13**	205	Sensitivity	**97.0**
Test Negative	**6**	**317**	323	Specificity	**96.1**
	198	330			

OraQuick

	Gold Standard: True Positive	Gold Standard: True Negative			
Test Positive	**198**	**0**	198	Sensitivity	**100**
Test Negative	**0**	**330**	330	Specificity	**100**
	198	330			

Stat-Pak

	Gold Standard: True Positive	Gold Standard: True Negative			
Test Positive	**198**	**0**	198	Sensitivity	**100**
Test Negative	**0**	**330**	330	Specificity	**100**
	198	330			

SureCheck

	Gold Standard: True Positive	Gold Standard: True Negative			
Test Positive	**198**	**1**	199	Sensitivity	**100**
Test Negative	**0**	**329**	329	Specificity	**99.7**
	198	330			

Uni-Gold

	Gold Standard: True Positive	Gold Standard: True Negative			
Test Positive	**198**	**1**	199	Sensitivity	**100**
Test Negative	**0**	**329**	329	Specificity	**99.7**
	198	330			

Table 3. Parallel Algorithms

Two test run simultaneously		Tie Breaker Test	Sensitivity	Specificity	No. of Times Tie Breaker Required	Cost (US$)*
Bundi	**Double Ch G**	**Determine**	100.0	99.4	**6**	**2.14**
		Stat-Pak	100.0	99.7		
		SureCheck	100.0	99.7		
		Uni-Gold	100.0	99.7		
Bundi	**Stat-Pak**	**Determine**	100.0	99.7	**1**	**2.85**
		Double Ch G	100.0	99.7		
		SureCheck	100.0	100.0		
		Uni-Gold	100.0	100.0		
Bundi	**SureCheck**	**Determine**	100.0	99.7	**2**	**3.25**
		Double Ch G	100.0	99.7		
		Stat-Pak	100.0	100.0		
		Uni-Gold	100.0	100.0		
Bundi	**Uni-Gold**	**Determine**	100.0	99.4	**2**	**3.10**
		Double Ch G	100.0	99.7		
		Stat-Pak	100.0	100.0		
		SureCheck	100.0	100.0		
Bundi	**Determine**	**Double Ch G**	100.0	99.4	**6**	**2.35**
		Stat-Pak	100.0	99.7		
		SureCheck	100.0	99.7		
		Uni-Gold	100.0	99.4		
Determine	**Double Ch G**	**Bundi**	100.0	99.4	**10**	**1.49**
		Stat-Pak	100.0	99.4		
		SureCheck	100.0	99.4		
		Uni-Gold	100.0	99.1		
Determine	**Stat-Pak ****	**Bundi**	100.0	99.7	**7**	**2.20**
		SureCheck	100.0	100.0		
		Uni-Gold	100.0	99.7		
		Double Ch G	100.0	99.4		
Determine	**SureCheck**	**Bundi**	100.0	99.7	**8**	**2.60**
		Double Ch G	100.0	99.4		
		Stat-Pak	100.0	100.0		
		Uni-Gold	100.0	99.7		
Determine	**Uni-Gold**	**Bundi**	100.0	99.4	**6**	**2.45**
		Double Ch G	100.0	99.1		
		Stat-Pak	100.0	99.7		
		SureCheck	100.0	99.7		
Double Ch G	**Stat-Pak**	**Bundi**	100.0	99.7	**7**	**1.99**
		Determine	100.0	99.4		
		SureCheck	100.0	100.0		
		Uni-Gold	100.0	100.0		
Double Ch G	**SureCheck**	**Bundi**	100.0	99.7	**8**	**2.39**
		Determine	100.0	99.4		
		Stat-Pak	100.0	100.0		
		Uni-Gold	100.0	100.0		
Double Ch G	**Uni-Gold**	**Bundi**	100.0	99.7	**8**	**2.24**
		Determine	100.0	99.1		
		Stat-Pak	100.0	100.0		
		SureCheck	100.0	100.0		
Stat-Pak	**SureCheck**	**Bundi**	100.0	100.0	**1**	**3.10**
		Determine	100.0	100.0		
		Double Ch G	100.0	100.0		
		Uni-Gold	100.0	100.0		
Stat-Pak	**Uni-Gold**	**Bundi**	100.0	100.0	**1**	**2.95**
		Determine	100.0	99.7		
		Double Ch G	100.0	100.0		
		SureCheck	100.0	100.0		
SureCheck	**Uni-Gold**	**Bundi**	100.00	100.00	**2**	**3.35**
		Determine	100.00	99.70		
		Double Ch G	100.00	100.00		
		Stat-Pak	100.00	100.00		

* Assuming the tie breaker test is required about 1% of the time.

** These are the first two tests used in the current test algorithm in Nigeria.

Table 4. Serial Algorithms

First Test	Second Test	Tie Breaker Test	Sensitivity	Specificity	No. of Times Tie Breaker Required	Cost (US$)*
Determine	**Bundi**	**Double Ch G**	100.0	99.4	**6**	**1.00**
		Stat-Pak	100.0	99.7		
		SureCheck	100.0	99.7		
		Uni-Gold	100.0	99.4		
Bundi	**Determine**	**Double Ch G**	100.0	99.7	**0**	**1.59**
		Stat-Pak	100.0	99.7		
		SureCheck	100.0	99.7		
		Uni-Gold	100.0	99.7		
Determine	**Double Ch G**	**Bundi**	100.0	99.4	**5**	**0.91**
		Stat-Pak	100.0	99.4		
		SureCheck	100.0	99.4		
		Uni-Gold	100.0	99.1		
Double Ch G	**Determine**	**Bundi**	100.0	99.4	**5**	**0.73**
		Stat-Pak	100.0	99.4		
		SureCheck	100.0	99.4		
		Uni-Gold	100.0	99.4		
Determine	**Stat-Pak**	**Bundi**	100.0	99.7	**7**	**0.99**
		Double Ch G	100.0	99.4		
		SureCheck	100.0	100.0		
		Uni-Gold	100.0	99.7		
Stat-Pak	**Determine**	**Bundi**	100.0	100.0	**0**	**1.44**
		Double Ch G	100.0	100.0		
		SureCheck	100.0	100.0		
		Uni-Gold	100.0	100.0		
Determine	**SureCheck**	**Bundi**	100.0	99.7	**7**	**1.03**
		Double Ch G	100.0	99.4		
		Stat-Pak	100.0	100.0		
		Uni-Gold	100.0	99.7		
SureCheck	**Determine**	**Bundi**	100.0	100.0	**1**	**1.84**
		Double Ch G	100.0	100.0		
		Stat-Pak	100.0	100.0		
		Uni-Gold	100.0	100.0		
Determine	**Uni-Gold**	**Bundi**	100.0	99.4	**6**	**1.01**
		Double Ch G	100.0	99.1		
		Stat-Pak	100.0	99.7		
		SureCheck	100.0	99.7		
Uni-Gold	**Determine**	**Bundi**	100.0	99.7	**0**	**1.69**
		Double Ch G	100.0	99.7		
		Stat-Pak	100.0	99.7		
		SureCheck	100.0	99.7		
Bundi	**Double Ch G**	**Determine**	100.0	99.7	**0**	**1.66**
		Stat-Pak	100.0	99.7		
		SureCheck	100.0	99.7		
		Uni-Gold	100.0	99.7		
Double Ch G	**Bundi**	**Determine**	100.0	99.4	**6**	**0.79**
		Stat-Pak	100.0	99.7		
		SureCheck	100.0	99.7		
		Uni-Gold	100.0	99.7		
Bundi	**Stat-Pak**	**Determine**	100.0	99.7	**1**	**1.64**
		Double Ch G	100.0	99.7		
		SureCheck	100.0	100.0		
		Uni-Gold	100.0	100.0		
Stat-Pak	**Bundi**	**Determine**	100.0	100.0	**0**	**1.50**
		Double Ch G	100.0	100.0		
		SureCheck	100.0	100.0		
		Uni-Gold	100.0	100.0		
Bundi	**SureCheck**	**Determine**	100.0	99.7	**1**	**1.68**
		Double Ch G	100.0	99.7		
		Stat-Pak	100.0	100.0		
		Uni-Gold	100.0	100.0		

Table 4. Serial Algorithms (continued)

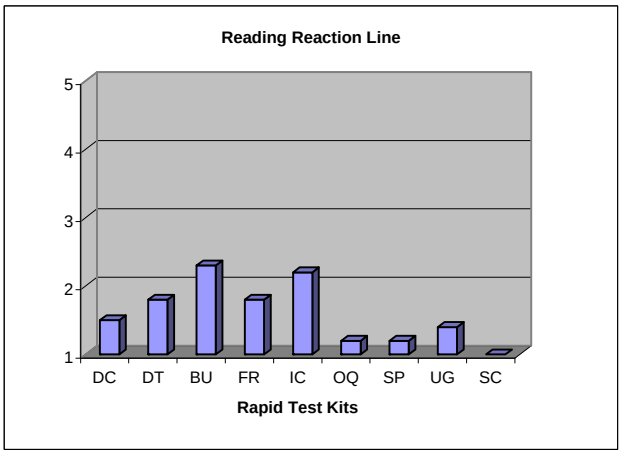
First Test	Second Test	Tie Breaker Test	Sensitivity	Specificity	No. of Times Tie Breaker Required	Cost (US$)*
SureCheck	**Bundi**	**Determine**	100.0	100.0	**1**	**1.90**
		Double Ch G	100.0	100.0		
		Stat-Pak	100.0	100.0		
		Uni-Gold	100.0	100.0		
Bundi	**Uni-Gold**	**Determine**	100.0	99.7	**1**	**1.66**
		Double Ch G	100.0	99.7		
		Stat-Pak	100.0	100.0		
		SureCheck	100.0	100.0		
Uni-Gold	**Bundi**	**Determine**	100.0	99.7	**1**	**1.75**
		Double Ch G	100.0	100.0		
		Stat-Pak	100.0	100.0		
		SureCheck	100.0	100.0		
Double Ch G	**Stat-Pak**	**Determine**	100.0	99.4	**7**	**0.78**
		Bundi	100.0	99.7		
		SureCheck	100.0	100.0		
		Uni-Gold	100.0	100.0		
Stat-Pak	**Double Ch G**	**Determine**	100.0	100.0	**0**	**1.41**
		Bundi	100.0	100.0		
		SureCheck	100.0	100.0		
		Uni-Gold	100.0	100.0		
Double Ch G	**SureCheck**	**Determine**	100.0	99.4	**7**	**0.82**
		Bundi	100.0	99.7		
		Stat-Pak	100.0	100.0		
		Uni-Gold	100.0	100.0		
SureCheck	**Double Ch G**	**Determine**	100.0	100.0	**1**	**1.81**
		Bundi	100.0	100.0		
		Stat-Pak	100.0	100.0		
		Uni-Gold	100.0	100.0		
Double Ch G	**Uni-Gold**	**Determine**	100.0	99.4	**7**	**0.80**
		Bundi	100.0	99.7		
		Stat-Pak	100.0	100.0		
		SureCheck	100.0	100.0		
Uni-Gold	**Double Ch G**	**Determine**	100.0	99.7	**1**	**1.66**
		Bundi	100.0	100.0		
		Stat-Pak	100.0	100.0		
		SureCheck	100.0	100.0		
Stat-Pak	**SureCheck**	**Determine**	100.0	100.0	**0**	**1.53**
		Bundi	100.0	100.0		
		Double Ch G	100.0	100.0		
		Uni-Gold	100.0	100.0		
SureCheck	**Stat-Pak**	**Determine**	100.0	100.0	**1**	**1.89**
		Bundi	100.0	100.0		
		Double Ch G	100.0	100.0		
		Uni-Gold	100.0	100.0		
Stat-Pak	**Uni-Gold**	**Determine**	100.0	100.0	**0**	**1.51**
		Bundi	100.0	100.0		
		Double Ch G	100.0	100.0		
		SureCheck	100.0	100.0		
Uni-Gold	**Stat-Pak**	**Determine**	100.0	99.7	**1**	**1.74**
		Bundi	100.0	100.0		
		Double Ch G	100.0	100.0		
		SureCheck	100.0	100.0		
SureCheck	**Uni-Gold**	**Determine**	100.0	100.0	**1**	**1.91**
		Bundi	100.0	100.0		
		Double Ch G	100.0	100.0		
		Stat-Pak	100.0	100.0		
Uni-Gold	**SureCheck**	**Determine**	100.0	100.0	**1**	**1.78**
		Bundi	100.0	100.0		
		Double Ch G	100.0	100.0		
		Stat-Pak	100.0	100.0		

*** Assuming a prevalence of 10% at testing sites.**

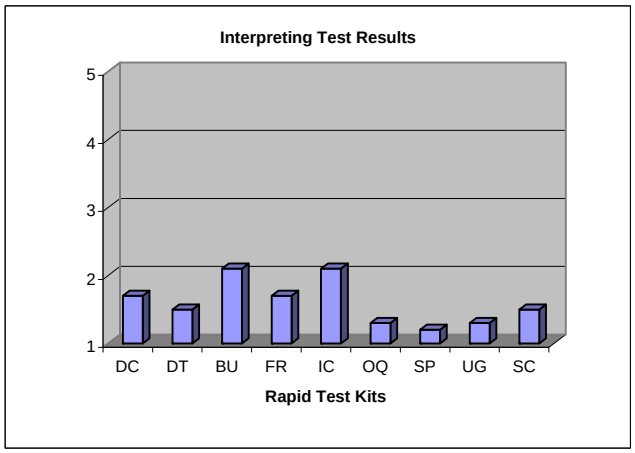
Figure 1. Results from Questionnaires Administered to Evaluation Testers

Questionnaire ratings have been averaged and graphically represented to facilitate a visual camparison of the various products.

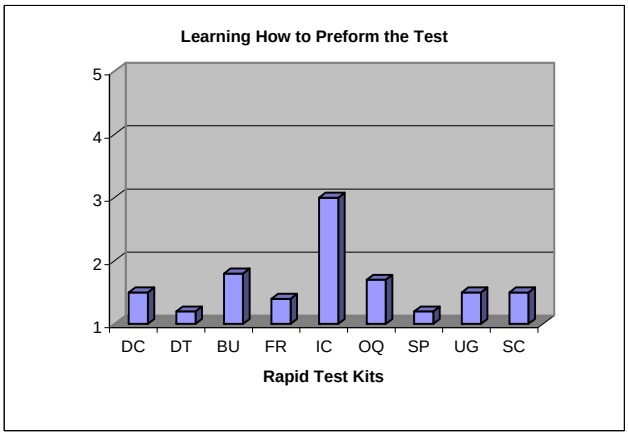
A.



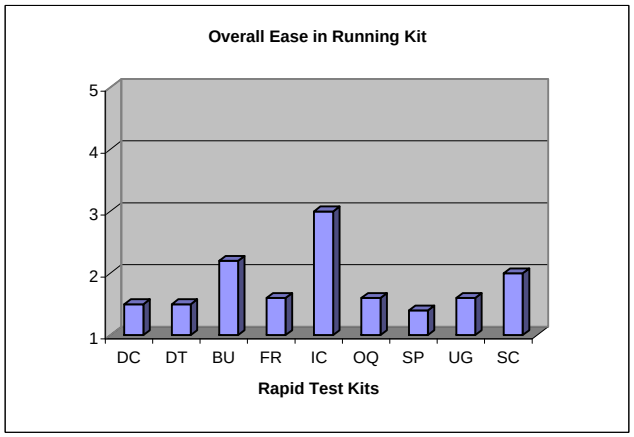
B.



C.

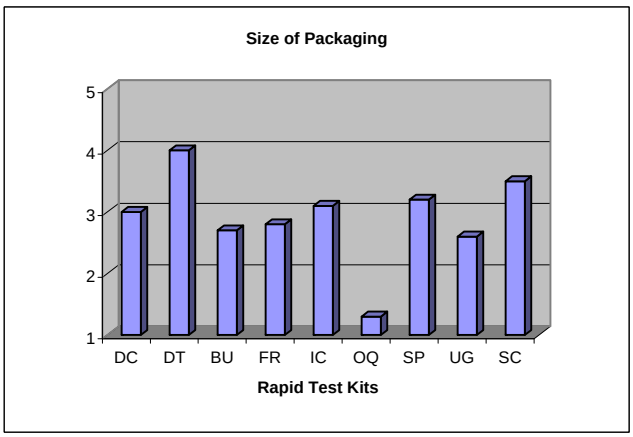


D.



Figures A-D: Testers were asked to rate various aspects of the rapid test kits on a scale of 1-5, 1 being the easiest and 5 being the most difficult.

E.



F.

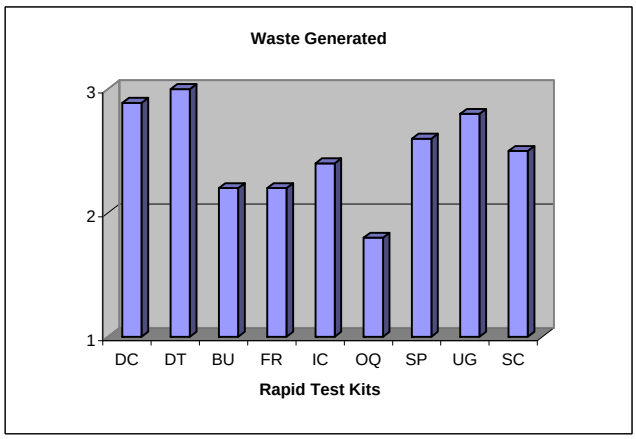


Figure E: Testers were asked to rate the size of the kit on a scale of 1-5, 1 being the most bulky and 5 being the most compact.
Figure F: Testers were asked to rate the amount of waste generated when running each test kit on a scale of 1-3,
1 being the most amount of waste and 3 being the least amount of waste.

Test kit abbreviations:
DC - Double Check, DT - Determine, BU - Bundi, FR - First Response, IC - InstantChek, OQ - OraQuick, SP - StatPak, UG - UniGold, SC - Sure Check

APPENDIX A.

Specimens for this evaluation were collected from the following sites:

Sero-conversion Project, Asokoro, Abuja
Plateau State Human Virology Research Centre
Plateau State Specialist Hospital, Jos
Aminu Kano Teaching Hospital
Nnamdi Azikiwe University Teaching Hospital, Nnewi
University of Benin Teaching Hospital, Benin
University of Nigeria Teaching Hospital, Enugu *
Usman Dan Fodio University Teaching Hospital, Sokoto *
University College Hospital, Ibadan *
University of Calabar Teaching Hospital, Calabar *
University of Maiduguri Teaching Hospital, Maiduguri *

* Specimens from these five sites did not arrive in time to be included in this evaluation.

Appendix B

Names and Contacts of Contributors

Technical Working Group Members

S/N	NAME	CONTACT ADDRESS
1	Ado Abubakar	CDC-Nigeria
2	Anyakora C. U.	NAFDAC, Abuja
3	Audu Israel Godwin	GHAIN
4	Dr. A.A.Adedeji	Epid Division, FMOH
5	Dr. Aminu S.M.	NBTS N/W Zone, Kaduna
6	Dr. Dauda Oladepo	NIPRD, Idu, Abuja
7	Dr. E. Akintunde	DOD-Nigeria
8	Dr. Issa B. Kawu	HIV/AIDS Division, FMOH
9	Dr. Njoku Moses	CBGE University of Jos
10	Dr.Akudo Ikpeazu	NACA
11	Dr.Ali Onoja	African Health Project
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13	Lt. (NN) L. Ukachukwu	NMOD-EPIC
14	Manason Rubainu	UATH Gwagwalada.
15	Miss.F. Simon	HIV/AIDS Division, FMOH
16	Mr. A. Abayomi	NBTS, Abuja
17	Mr. Adonye T.O.	HIV/AIDS Division, FMOH
18	Mr. Asukwo Uwah	HIV/AIDS Division, FMOH
19	Mr. G. Ikwulono	HIV/AIDS Division, FMOH
20	Mr. Idris Saliu	Safe Blood for Africa Foundation
21	Mr. Joseph Jugu	IHV-Nigeria, Abuja
22	Mr. Yakubu Kachiro	HIV/AIDS Division, FMOH
23	Mrs Bassey G. M.	FMOH
24	Nwadike P. Jean	IHV-Nigeria
25	Oke, Odafen	CDC -Nigeria
26	Patrick Ikani	GHAIN
27	Prof. A.H. Fagbami	WHO Consultant
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Laboratorians who carried out the evaluation

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4	Frank Agbontaen	UBTH, Benin
5	Hamisu Takalmawa	AKTH, Kano
6	Idowu D. Bose	UDUTH, Sokoto
7	Kayode Emmanuel	Asokoro General Hospital Abuja
8	Patience Amangam	PLASVIREC, Jos
9	Simon Esiet	UPTH, PortHarcourt
10	Stephen Kalu	NAUTH, Abia State

Other contributors

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3	Dr.O. Salawu	FMOH
4	Prof. D. Olaleye	UCH, Ibadan
5	Ado Abubakar	CDC-Nigeria
6	Bimpe Okelade	IHV-Nigeria
7	Oke Odafen	CDC-Nigeria
8	Orji Bassey	CDC, Nigeria
9	Tapdiyel D. Jelpe	CDCD-Nigeria

APPENDIX C.

Data Collection Sheet (Nigeria HIV Rapid Test Evaluation)

Name of Rapid Test: _____

Date Tested (& time of day): _____

Kit Lot: _____

Kit Exp Date: _____

Lab Tech: _____

Reporting Codes
Please use the codes below for reporting test results.
P = Positive
N = Negative
I = Invalid
Ins = Insufficient Sample Volume

Pos and Neg Controls Worked: | **Yes** | **No**

Room temperature Reading:

Specimen ID#	Test Result	Specimen ID#	Test Result	Specimen ID#	Test Result	Specimen ID#	Test Result

Comments:

APPENDIX D.

TESTERS' RATINGS OF RAPID TEST KITS (RTKs) DURING LABORATORY EVALUATION

Instructions:
The purpose of this questionnaire is to find out how <u>you</u> rate the RTKs currently under lab evaluation.
You will be given this questionnaire after you have evaluated each rapid test.
You do not need to put your name on the form.
Please be open and honest. Along with other information, your feedback on this questionnaire will help form decisions on which RTKs will be recommended for use in Nigeria.
Please take 5-10 minutes to complete this form.
After completing the form, please give it to one of the lab supervisors.

1. What test kit did you just run?

_____

Please rate each of the RTKs on the following criteria by circling the most appropriate response using this scoring system:

1	2	3	4	5
Very easy	Easy	Neither	Difficult	Very Difficult

2. Collecting and delivering the **correct volume of plasma/sera** onto the device:

1 2 3 4 5

3. Adding **diluent/ wash/ chase buffer** correctly onto the device:

1 2 3 4 5

4. Reading the test result within the **correct time period**:

1 2 3 4 5

5. Reading the test result (was it easy or difficult to **read the lines, was the line dark enough**):

1 2 3 4 5

6. Interpreting the test (deciding whether the test positive/ negative based on lines or clumping):

1 2 3 4 5

7. Learning how to perform the test (was it easy for you to learn how to perform this test, would it be easy to train others how to perform this test):

1 2 3 4 5

8. Overall ease of use:

1 2 3 4 5

9. Design of the test device for writing patient ID number (was it easy for you write the ID number, was adequate space provided):

1 2 3 4 5

10. How often did you obtain an invalid test result (test control line not present or no results were generated): ***Please state number of invalid test results you got during the testing period. If none, please write 0.***

I had _____ invalid tests out of a total of _____ specimens.

11. Did you find any **defective test devices or accessory supplies?** ***Report how many or the total number of specimens tested***

I found _____ defective tests while testing _____ specimens.

12. Were there any problems **with any of the RTKs during the study period (in particular around ease of leaning how to use the test, how to perform the test and how to interpret the test)?**

_____

_____

_____

_____

13. Would you recommend the use of this test kit, if <u>NOT</u>, give all your reasons?
***Please list all of the reason(s) that apply*.**

_____

_____

_____

14. What is your opinion of the test kit packaging? ***Rate each aspect by circling one answer:***

What did you think of the **size of the test kit box/package**?

1	2	3	4	5
Very Bulky	Bulky	Moderate	Compact	Very Compact

How **roughed is the packaging**?

1	2	3	4	5
Very Flimsy	Flimsy	OK	Robust	Very Robust

How much **waste** was generated in running your set of specimens?

1	2	3
Very Much Waste	Much Waste	Minimal Waste